

With compliments to
Professor Marshall

19


ON THE
ACTION OF A SECRETION OBTAINED FROM THE
MEDICINAL LEECH ON THE COAGULATION
OF THE BLOOD:

BY

J. B. HAYCRAFT, M.B., F.R.S. (Edin.),

Professor of Physiology in the Mason and Queen's Colleges, Birmingham.

[From the PROCEEDINGS OF THE ROYAL SOCIETY, No. 231, 1884.]



“On the Action of a Secretion obtained from the Medicinal Leech on the Coagulation of the Blood.” By JOHN B. HAYCRAFT, M.B., F.R.S. (Edin.), Professor of Physiology in the Mason and Queen’s Colleges, Birmingham. Communicated by Dr. LAUDER BRUNTON, F.R.S. Received March 28, 1884.

The following is an offshoot from a more extended investigation upon the coagulation of the blood not yet ready for publication.

The blood flowing from a leech-bite is not readily stopped, often flowing for upwards of an hour after the animal has been removed. The blood within the body of the creature remains fluid for an indefinite time; and when ejected it is found to have lost its coagulability. These are facts known to every surgeon, but they have received no explanation.

While endeavouring to solve one very difficult problem, “why the blood does not coagulate within the living blood-vessels,” the above facts came to my mind and promised to throw some light on the general question.

The explanation which at once suggested itself to me was, that probably the leech secretes some ferment-containing-juice which antagonises the blood ferment, preventing coagulation within its body, enough remaining around the edges of the wound to prevent for some time the outflowing blood from clotting; the blood remaining fluid, in fact, until the leech ferment is all washed away.

It will be seen that this explanation is in the main correct.

In order to investigate its action on the blood, a salt solution extract of leech was obtained. In the first experiment the gullets and buccal cavities of two leeches were removed, cut in small pieces, and placed in 5 cub. centims. of 6 per cent. salt solution. The rest of the alimentary canals were also finely divided and placed in a similar volume of the salt solution. The extracts obtained were of a faint greenish-yellow tint, and alkaline in reaction. A leech was then applied to the nose of a young rabbit for a quarter of an hour, then removed, and held in a salted towel until it had disgorged. Meanwhile the blood flowing from the nose of the rabbit was collected in a test-tube.

The rabbit was then killed, and 3 cub. centims. of its blood received

into each of four vessels A, B, C, and D, which were placed under a glass shade, the air within being kept moist with blotting-paper soaked in water.

Vessel A contained nothing but the blood, which coagulated in four minutes.

Vessel B contained in addition 1 cub. centim. of 6 per cent. salt solution, and coagulated in four minutes.

Vessel C contained in addition 1 cub. centim. of 6 per cent. salt solution of the alimentary canals (minus the pharynx) of the leeches. The blood faintly coagulated in four minutes, setting completely in half an hour.

Vessel D contained in addition 1 cub. centim. of 6 per cent. salt solution extract of gullet of leeches. This remained fluid for twenty-four hours, a thick clotted scum forming on the surface when the blood putrefied.

The blood collected from the nose of the rabbit and that from the interior of the leech remained fluid until putrefaction set in, when the same half scum half clot formed on the surface.

It is evident from this experiment that something retarding or preventing coagulation may be extracted with 6 per cent. salt solution from the pharynx or mouth of the leech.

The slight action of the rest of the alimentary canal in retarding coagulation, as this and many subsequent experiments indicate, is due to some of the secretion diffusing downwards. Its place of origin within the body of the leech is certainly the pharynx or buccal cavity, decoctions of which are more than ten times as strong as those of the rest of the body.

The next experiment was undertaken to test my supposition that this substance belonged to the class of ferments, the most general character of which is that they are destroyed by boiling. At the same time solutions of the leech in distilled water were made in order further to test its solubility.

Equal portions of the blood of a sheep were placed in four vessels containing equal volumes of leech extract.

Vessel A contained salt solution extract of gullet of leech; the blood remained fluid for three hours and was not further observed.

Vessel B contained distilled water extract of gullet; the blood remained fluid for a like period.

Vessel C contained *boiled* salt solution extract; the blood remained fluid for a like period.

Vessel D contained *boiled* distilled water extract; the blood remained fluid for a like period.

The blood received in a test-tube from the body of the animal coagulated in five minutes. This experiment was repeated with similar results.

We have then to do with a substance not a ferment, and soluble in distilled water, as well as in salt solution.

It may be noted that when the leech extracts are added to blood the corpuseles subside to the bottom of the vessel, leaving, in the case of the salt solution extract, a clear plasma, and in the case of the watery extract a plasma coloured by the hæmoglobin, which is partly dissolved.

In order to obtain the active principle of the leech extract in a pure form an attempt was made to isolate it by means of the ordinary solvents.

A watery extract of several leeches was evaporated to dryness, and divided into portions which were respectively extracted for twelve hours with chloroform, ether, benzole, and absolute alcohol. They were then filtered, and the filtrates evaporated to dryness and extracted again with water. They had no action whatever on blood, except in the case of the chloroform extract, and that was very slight indeed.

The residues after extraction with these fluids were dissolved in water, and proved efficacious when added to blood.

The substance is then insoluble in chloroform, ether, benzole, and alcohol.

Its separation in a pure form being evidently a difficult matter owing to its insolubility in the most useful solvents, the attempts at its isolation were in the meanwhile abandoned.

One useful result of this experiment was the preparation of a much purer solution to be used for experimental purposes. The gullets of the leeches were placed in absolute alcohol for a day or two, and then extracted with water. In this way a clear, somewhat coloured alkaline fluid was obtained, almost free from albumen, and giving but a very small residue, this, too, of unimpaired strength. A solution prepared in this way was henceforth always used.

The next task was to find the exact action of this substance on the blood, and in the first case to see if it destroyed or hindered the action of the ferment.

After trying in vain one or two methods the following suggested itself:—

The blood-coagulating ferment is not very soluble in distilled water, so that if a blood-clot containing it be soaked for some time in the watery leech extract, this can afterwards be removed by distilled water without dissolving out the ferment. On adding the latter to hydrocele fluid its activity can be tested (this fluid coagulates on the addition of active blood ferment).

The washed finely divided clot from bullocks' blood was dried and divided into two equal portions by weight. One portion was then placed in a watery solution of leech, and the other in a like volume of distilled water. After twelve hours the clots were removed, dried

with blotting-paper, and carefully washed in distilled water. The washing lasted for three hours, the wash water being four times renewed, each portion of clot being treated with the same volume of water. They were then each placed in a few cubic centimetres of 8 per cent. salt solution in which the blood-coagulating ferment is soluble. Five drops of the salt solution extract of the clots were added to equal quantities (5 cub. centims.) of hydrocele fluid. The hydrocele fluid to which the extract of clot, treated with leech extract, had been added did not coagulate at all, while the other portion coagulated in ten minutes.

The action of the leech extract is then seen to destroy the blood-coagulating ferment.

The next question is whether the blood corpuscles are in any way affected by the leech extract? A couple of drops of blood were obtained from the finger-tip, mixed rapidly with a drop of normal salt solution extract of leech, and a minute drop of the mixture placed on a glass slide. This was then covered, and a ring of oil was painted round the edge of the cover-glass to prevent evaporation. When this was examined on a warm stage, the red corpuscles rapidly formed roulcaux in the ordinary way; and the white corpuscles exhibited active amœboid movements which continued for a considerable time—the observations lasted for an hour. In the meanwhile the blood had not coagulated. It is seen then that the vitality of the white corpuscles is not destroyed, and that the formation of roulcaux by the red disks is not necessarily a phenomenon of coagulation. The coagulation of the perivisceral fluid of the sea urchin is, according to the observations of my friend Mr. Patrick Geddes, not due to the intercellular formation of fibrin in the plasma, but to active amœboid movements of the white corpuscles. The pseudopodia of neighbouring cells join, become welded together, and contract so that all the cells are massed together into a solid clot. Professor Schäfer maintains that there is also a formation of fibrin in the ordinary way which has been overlooked by Mr. Geddes.* I have not examined this fluid myself, and do not feel qualified to speak upon this subject; but certainly the blood of crabs, crayfish, and lobsters clots in the way described by the former of these authors, there being no intercellular formation of fibrin. In this case, then, the leech extract will probably not prevent coagulation. The blood of a crayfish was mixed with one-third of its volume of leech extract. A drop was examined microscopically at the ordinary temperature. The cells exhibited normal amœboid movements, and coagulation was apparently quite normal.

Heretofore we have spoken of the extract of leech gullet and buccal cavity, but it has not yet been shown which is the source of

* "Proc. Roy. Soc.," vol. 34, p. 370.

this excretion. All that can be said is that it comes from either the gullet or mouth and sucker of the animal. Careful microscopical preparations were made of the anterior half of the leech, to seek for glandular structures. The animals were hardened for ten hours in saturated picric acid solution, and longitudinal mesial sections were made with a freezing microtome. The sections were stained with picrocarmine. No signs of ordinary glandular structures were to be observed either opening into the sucker, or into the alimentary canal. Some of the epithelial cells forming the integument of the leech are much elongated, passing down even among the subjacent muscular fibres. These have been looked upon as unicellular glands (Ray-Lankester), and in the sucker one may see small collections of these. If the skin lining the sucker be removed, it is found to be active in preventing coagulation. Not in a less degree however is the anterior part of a leech from which the skin of the sucker has been removed. Probably then the secretion is derived from the epithelial cells lining the sucker and buccal cavity; it may be that the unicellular glands of the sucker share in its production.

The products of the digestion of albumin by pepsin have an important action in retarding coagulation. Dr. Schmidt-Mulheim,* working under the direction of Professor Ludwig, has shown that blood received from an open vein into a solution of peptone does not coagulate with its normal rapidity, being retarded for nine or ten minutes. If the peptone solution be injected into the veins of a dog, and blood withdrawn from the animal at intervals after the injection, it will, if the injection be powerful enough (.3 grm. peptone to every 1000 grms. body weight of animal), materially affect its coagulability.

Dr. Fano† finds that certain products of tryptic digestion also retard coagulation in dogs; but curiously enough neither digestion products have any action on rabbits. It may be mentioned that when injected into dogs in larger quantities the blood pressure falls, there being great determination of blood to the abdomen and visceral veins; convulsions are observed, and the animal dies in a state of coma, the blood found within the body being of course fluid.

The coagulability of the blood produced by certain poisons is altogether a question of great interest; for instance, most snake bites (the cobra is an exception) produce permanent fluidity of the blood, and we know nothing either of the relation of that change to concomitant symptoms, or the specific action of the venom on the blood itself.

Now inasmuch as the extract of leech is much more powerful than the peptone solution in preventing coagulation outside the

* "Archiv. für Phys.:" Du Bois-Reymond, 1880, p. 33.

† "Archiv. für Phys.," 1881, p. 276.

body, its effects when injected into the system may naturally be expected to lead to striking results.

The difficulties in obtaining a license to perform the experiments in England being very great, I availed myself of an invitation from my friend Professor Schmiedeberg, of Strasburg, who kindly placed his laboratory at my service. It is with the greatest pleasure that I remember his kindness and courtesy, I wanted nothing that his well-organised laboratory contained, and to him I owe much personal assistance in an attempt to isolate the active principle present in the leech's pharynx. This attempt, although not as yet successful on account of the great difficulty of the task, will I hope at some future time be renewed with more fruitful result.

In the following experiments the extract of leech was prepared by placing the anterior part of the animal in absolute alcohol for three days, then evaporating off the alcohol, grinding the leech with broken glass, extracting with water and filtering.

Experiment with a Dog weighing 5000 grms.—Blood withdrawn by means of a cannula placed in the carotid artery coagulated in 3 minutes.

Temperature $39^{\circ}3$ (rectum).

5.22 P.M. 20 cub. centims. solution of 8 leeches injected into left jugular vein.

5.25 P.M. 1 cub. centim. of blood withdrawn. It remained fluid until the next morning, never completely coagulating.

5.45 P.M. 1 cub. centim. of blood withdrawn, which remained fluid until morning.

6.5 P.M. 1 cub. centim. of blood withdrawn, which remained partly fluid till morning.

6.25 P.M. 1 cub. centim. of blood withdrawn, which coagulated in 25 minutes.

7.0 P.M. 1 cub. centim. of blood withdrawn, coagulated in 5 minutes.

During this experiment the respirations were somewhat increased in number, and the temperature rose gradually until it reached $40^{\circ}1$ at 7 o'clock.

In the case of peptone injection a marked difference between its effects on dogs and on rabbits is seen. A rabbit was therefore next chosen for the subject of an experiment. A cannula was inserted into the carotid for the purpose of withdrawing blood at intervals.

1 cub. centim. of blood withdrawn. Coagulated in 0.8 minute.

4.40 P.M. 5 cub. centims. of solution from 5 leeches injected into left jugular vein.

4.45 P.M. 1 cub. centim. of blood withdrawn from the carotid. It remained fluid for 12 hours.

4.47 P.M. 1 cub. centim. of blood withdrawn from the carotid. A moiety coagulated in 2 hours.

5.20 P.M. 1 cub. centim. of blood withdrawn. In 25 minutes almost completely clotted.

5.40 P.M. 1 cub. centim. of blood withdrawn. In 10 minutes it clotted fast.

6.0 P.M. 1 cub. centim. of blood withdrawn. In 3 minutes it clotted fast.

In my first experiments when an animal was allowed to live after the operation, the wound in the neck was stitched up in the ordinary way. When examined next morning a large swelling was always observed in the region of the wound. On observing these they were found to consist of imperfectly clotted blood which had escaped from the divided capillaries, the fluid unable to clot had continued to collect often in very large amount, passing down under the skin in front of the chest, and often causing death. Subsequently the wound was carefully painted with perchloride of iron before sewing it up, and no after-bleeding occurred. These facts are not without interest, as they throw light upon a similar symptom seen in many cases of hæmophilia or hæmorrhagic diathesis, where the slightest wound gives rise often to very troublesome bleeding.

It was found by experiment that the injection of the leech extract has no immediate action on blood pressure. After the injection of the substance the animals as a rule appeared somewhat dull, the respirations were somewhat increased and the temperature elevated, but they soon recovered even after injection of large quantities; in one case a rabbit weighing only 1080 grms. was hardly disturbed by an injection of an extract of twelve leeches.

The action of the leech extract, like that of the digestion products of Dr. Schmidt-Mülheim, is not permanent. In a few hours the blood is again normal. This may be from various possible reasons, either that more and more blood ferment is excreted, overpowering as it were the action of the leech extract, or that the latter is oxidised and broken up in the tissues, or finally that it is excreted (probably with the urine). This latter surmise was from the first held by my accomplished friend, Dr. Kobert, and the following experiments show it to be true.

The urine was withdrawn from the dog used in the last experiment $1\frac{1}{2}$ hours after the injection of the leech extract. At the same time urine was obtained from a healthy animal. Two drops of each fluid were added to two equal portions (7 drops) of frog's blood. Whereas the one mixed with healthy urine coagulated in 10 minutes, the other remained fluid for more than 12 hours. The experiment was repeated with a like result.

Before leaving the action of the leech extract on the blood, it may

be well to contrast its action with the peptone injection of Schmidt-Mulheim and Fano. In the first place, I should be inclined to doubt whether the active substance in these investigations is peptone at all. It was found that after peptone had ceased to give any reaction in the blood, it was still uneoagulable; the conclusion of Dr. Fano being that it forms some compound with other blood constituents which prevents the coagulation. Is it not more natural to suppose that peptone has nothing to do with the question at all, especially as there are many other digestion products difficult to separate from the peptone. Suppose the same substance formed in the leech were present—which it is not—in the digestion products, it would not have been separated from the peptone prepared by these observers. At any rate, the action of the injection, whatever it is, shows a marked difference in its results from the leech extract. The latter produces far milder constitutional symptoms, is far more powerful in its action on the blood, and has the same action on rabbits as on dogs.*

Since my return to my own laboratory I have investigated the action of the leech extract on the coagulation of milk by rennet and on myosin. Rennet mixed with a watery solution of leech has no less power of clotting milk than another portion mixed with water alone. The leech extract does not hinder in any way the clotting of myosin. The latter was prepared from frog's muscle in a somewhat shorter and, I think, a better way than that described by Kühne. Two mortars containing pounded glass were placed in a freezing mixture. One held a few drops of normal salt solution, and the other a like quantity of normal salt solution of leech extract. The legs of a frog were washed out with normal salt solution, the muscles cut out, dried on blotting-paper, placed in the mortar, and ground with the glass into a frozen pulp. This was then tied up in a piece of calico, and the myosin expressed and filtered. Its preparation is most easy, and can be done without fail in the hottest weather. The myosin obtained from the muscle to which the leech extract had been added coagulated in 10—15 minutes, and the other in from 20—25 minutes. The experiment was repeated with like results. It may be remarked that if the clotting of myosin is due to a ferment, this has not yet been isolated.

Inasmuch as during contraction chemical changes occur very similar to those seen in rigor mortis, and inasmuch as in the latter

* My friend Dr. Wooldridge believes that in the coagulation of the blood one factor, at any rate, is the conversion of the white cells into fibrin by the dead plasma ("Proc. Roy. Soc.," vol. 31, p. 413). Without discussing the probability of such a view being correct or not, it certainly is not true in the clotting of the hydrocele fluid by an extract of clot (ferment). In this case my experiments show a special action of the leech extract on the ferment, which, when active, converts large portions of the plasma into fibrin.

the coagulation of myosin is a prominent fact, some have held that a contraction of a muscle is due also to a partial clotting of the myosin. This view I have never held to be anything but fanciful, for reasons which cannot be stated here. Dr. Lauder Brunton, however, suggested to me, that by soaking frog's muscle in the leech extract, and studying its contractility, some light might be thrown on the subject. I accordingly cut out from the limbs of a pithed frog some of the principal muscles, *i.e.*, the sartorii, carefully removing them with as little injury as possible. Those of one side were placed in normal salt solution, and those of the other in normal salt solution of leech. Comparisons were of course only made between the corresponding muscles of both sides. From time to time the irritability was tested by currents from an induction coil. In all cases the muscles placed in leech extract lost their irritability some time before the corresponding ones placed in salt solution. In one experiment with two sartorii, the left placed in leech extract died in 35 minutes, the right in 70 minutes. Two pectoral muscles attached to the bone lived much longer—one longer than I had conceived was possible—that in leech extract for 12 hours, and the other for 51 hours.

In these experiments those placed in leech extract almost from the first contracted more feebly when stimulated. On more than one occasion I obtained partial recovery when a muscle was removed from the leech extract and placed in fresh salt solution. The only conclusion one can draw is, that the leech extract somewhat hastens the coagulation of myosin removed from muscle, and probably when it causes loss of contractility in a muscle, it is due to the same reason, inducing, in fact, rigor mortis, the essential phenomenon of which is clotting of the myosin.

In conclusion, then, it may be stated that the leech secretes from its mouth a fluid which destroys the blood ferment without producing any other observable change in the blood. This injected into an animal produces but slight constitutional disturbance, and is eliminated by the kidneys. The action on the rabbit is the same as on the dog; on crustacean blood it is inert. It has no action on the curdling of milk, slightly hastening the clotting of myosin, and hastening rigor mortis.

